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THE SIGNIFICANCE OF STREPTOCOCCI IN MILK.*

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OF late years attention has been frequently called to the presence streptococci in milk. Von Lingelsheim[1] mentions the fact that streptococci are found in market milk, and suggests that these bacteria may be the cause of many cases of infantile intestinal diseases. Streptococci in milk have also been reported by Conn,[2] Conn and Esten,[3] Bergey,[4] Barthel,[5] Reed and Ward,[6] who also found them in the udder, and others, and special stress has been laid on the significance of leucocytes in market milk if streptococci are present. An examination by the writer of fresh milk of the highest character, in the summer of 1905 resulted in finding that streptococci are invariably present even in milk obtained with unusual precautions and from healthy cows.

This led to an investigation of the relation between the streptococci and the lactic acid bacteria ordinarily concerned in the souring of milk, it being known, among other things, that streptococci can produce and withstand a large amount of acid.

Pasteur[7] was the first to describe lactic acid bacteria. His "levure

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*Received for publication, January 5, 1906.

[1] KOLLE UND WASSERMANN, *Handbuch der pathogenen Mikroorganismen*, 1903, 3, p. 337.

[2] *Centralbl. f. Bakt.*, Abth. 2, 1902, 8, p. 442.

[3] *Ann. Report Storrs Agric. Exp. Station*, 1902-3, p. 63.

[4] *Dept. of Agriculture, Commonwealth of Penna., Bull. 125*, 1004, p. —.

[5] *Milchzeitung*, Leipzig, 1903, pp. 40, 41, 42.

[6] *Centralbl. f. Bakt.*, Abth. 1, 1903, 30, p. 83.

[7] *Ann. de chim. et phys.*, Série 3, 1858, 52, p. 404.

lactique" consisted of "small globules or very short particles resembling certain kinds of amorphous precipitates. They are constricted in the middle, occur isolated or in irregular clusters. They ferment lactose with acid and gas formation, and cultures are often viscid." In 1878 Lister[1] isolated an organism from sour milk by the dilution method which he called *Bacterium lactis*, a detailed description of which could not be found. In 1884 Hueppe[2] described his *B. acidi lactici*, which he found in large numbers in all samples of milk examined, and which was subsequently accepted as the lactic acid organism *par excellence*. This organism so far as can be gathered from the original description, is to be considered as a variety of *B.* [*lactis*] *aërogenes*, Escherich. Following this discovery many varieties of lactic acid bacteria have been described, some of which may be identified with the one discovered by Hueppe. Clauss,[3] Grotenfelt,[4] Schardinger,[5] Kozai(2)[6] and Utz (2),[7] have isolated lactic acid bacteria which apparently are identical with, or at most varieties of, *B. aërogenes*. The luxuriant growth on all media is characteristic. On agar, in ordinary broth, on potato, and in proteid-free media, there is abundant growth. Dextrose and lactose are fermented with acid and gas formation; milk is coagulated more or less rapidly, the coagulum contracts characteristically and whey is separated. Colonies on agar or gelatin are large, moist, and often viscid. Stab cultures produce good growth on the surface and in the puncture.

A wholly different class of lactic acid bacteria has been described by Leichmann,[8] Günther and Thierfelder,[9] Kozai (1),[10] Utz (1),[11] Esten,[12] Schierbeck,[13] and others. Günther and Thierfelder identify their organism erroneously with Hueppe's. These bacteria have been usually described as bacilli, but regarded as distinct from the *B. aërogenes* type. Leichmann was the first to call attention to this difference and unfortunately named his organism *B. lactis acidi* in distinction from *B. acidi lactici*, Hueppe. This appellation has been the cause of great confusion.

[1] *Quarterly Jour. of Microscopical Science*, 1878, 18, p. 177.

[2] *Mitth. a. d. kaiserl. Gesundh. Amt.*, 1884, 2, p. 309.

[3] *Inaug. Dissert.*, Würzburg, 1889.

[4] MIGULA, *System der Bakterien*, 1900, 2, p. 544.

[5] *Monatshefte f. Chemie*, 1890, 11, p. 544.

[6] *Ztsch. f. Hyg.*, 1899, 31, p. 337.

[7] *Centralbl. f. Bakt.*, Abth. 2, 1904, 11, pp.600 and 733.

[8] *Ibid.*, 1896, 2, p. 777.

[9] *Arch. f. Hyg.*, 1895, 15, p. 164.

[10] *Loc. cit.*

[11] *Loc cit.*

[12] *Ann. Rep. Storrs Agric. Exp. Station*, 1896.

[13] *Arch. f. Hyg.*, 1901, 38, p. 294.

The experiments reported in this paper comprise first a comparative study of cultural characteristics of the following organisms:

Four lactic acid bacilli, true bacilli, one of which was in the laboratory collection labeled *B. acidi lactici;* the other three were isolated from loppered milk. These all proved to be of the *B. aërogenes* type.

Eight lactic acid organisms received from the following laboratories under the name *B. acidi lactici:* One from Professor Russell, University of Wisconsin; three strains from Professor Harding, N. Y. Agric. Exp. Sta., Geneva, N. Y.; one from Professor Prescott, Mass. Institute of Technology, Boston, Mass.; one from Professor Marshall, Michigan Agricultural College; one from Professor Bouska, Ames Agricultural College, Iowa; and one from Professor Conn, Wesleyan University, Middletown, Conn. All of these were found to belong to the group *B. lactis acidi*, Leichmann.

In addition a streptococcus from Chicago milk, isolated in this laboratory by Dr. Harris in February, 1904, was obtained for this investigation in May, 1905, and a streptococcus isolated from sewage also by Dr. Harris. Five pathogenic streptococci isolated from cases of scarlet fever, tonsilitis, and phlegmon, by Dr. Weaver, Memorial Institute of Infectious Diseases, Chicago, in February, 1904, were received for this investigation in May, 1905.* Streptococci were then isolated by the writer from fresh milk, sour milk, separator slime, market milk, cow feces, and from drippings from the udder during the process of washing previous to milking. These latter agreed well with each other and also with the so-called *B. lactis acidi.* The experiments were carried on in duplicate, one set being kept at 37° C., the other at room temperature.† Except for the fact that growth, in the majority of cases, was somewhat better at 37° C. than at room temperature, no noteworthy difference was brought out by these different temperatures.

The bacilli of the *B. aërogenes* type are all negative to Gram's stain, are generally plump, oval, and in irregular clusters. Diplobacillus forms are not infrequent. Two have capsules, one is viscid in cultures, and gas is formed by all from dextrose and lactose. On agar and potato and in gelatin and non-proteid media, with and without lactose, growth is luxuriant. The colonies are well developed on the surface, are large, moist, and viscid. No difference from typical *B. aërogenes* could be noted.*

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*I wish to acknowledge my gratitude to those who have kindly supplied me with cultures for this work.

†Media were prepared according to the methods prescribed by the Bacteriological Committee of the Amer. Pub. Health Assoc. (*Jour. Infect. Dis.*, 1905, Supplm. No. 1) and 2 per cent lactose added to agar, broth, and gelatin. The formula recommended by Jordan (*Botan. Gazette*, 1899, 27, p. 1) was employed for non-proteid media. Bacteria were isolated from milk by the usual plate method with lactose-litmus agar.

A striking similarity was found to exist between bacteria of the streptococcus and the *B. lactis acidi,* Leichmann types. The characteristics of these are briefly as follows:

Morphology.—Cocci, sometimes oval, mostly in pairs, and often with pointed ends, frequently in chains of three to six members, and sometimes in chains of considerable length. Cultures in lactose broth showed chain formation to best advantage. The cocci are positive to Gram's stain.

Culture media.—Growth on slant agar and potato and in plain broth is uniformly poor, considerably better if lactose is added to media. On slant agar a delicate ribbon-like layer is formed, which often consists of single or confluent colonies, and in a few cases is hardly visible. On the surface of stab cultures there is a barely visible growth or none at all; in the puncture, however, it is quite fair. Coagulation of milk varies, from one to fourteen days being required. Litmus, if added to milk, is decolorized by *B. lactis acidi,* and all streptococci in the same typical manner. The solid coagulum turns white leaving a pink ring at the top, which gradually extends toward the bottom, and little or no whey is liberated. In non-proteid media there is no noticeable growth.

Colonies.—Small, irregular, appear mostly in the depths, but few on the surface.

The next inquiry concerned the variability of coagulative power. Experiments were conducted by passing each of the above-mentioned organisms, 33 in all, through eight tubes of litmus milk, successively. The milk employed was mixed previous to tubing and sterilizing, to insure uniform composition. Transfers were made every other day. The majority of streptococci, especially pathogenic ones, gained in coagulative power. The same was observed in streptococci from cow feces and sewage. The appearance of the coagulum of all streptococci and *B. lactis acidi,* Leichmann type was alike—showing the typical decolorization and the red ring on top.

The opinion of Houston,[1] that streptococci in water are of value in detecting sewage contamination, has received some support.[2] The method employed is to inoculate dextrose or lactose broth with definite amounts of the water in question. Bacteria of the Colon-Aërogenes group multiply rapidly, and when a certain amount of acidity is reached, streptococci, being able to resist a higher degree of acidity, crowd other bacteria out. Judging from analogy it is reasonable to assume that similar conditions obtain in milk. Three lines of research were carried on to establish this view.

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* The rules of nomenclature possibly demand that "*B. acidi lactis,* Hueppe" be adopted in place of *B. lactis aërogenes* on account of priority. As this would undoubtedly lead to confusion it seemed best for the present to retain the name "*B. aërogenes.*"

[1] *Supplm. to the 29th Annual Rep. of the Local Gov. Board,* containing the *Rep. of Med. Officer for 1899–1900,* p. 458.

[2] WINSLOW AND HUNNEWELL, *Jour. Med. Res.,* 1902, 3, p. 502; PRESCOTT AND BAKER, *Jour. Infect. Dis.,* 1904, 1, p. 193.

(1) One quart of certified milk was divided into two parts and placed in two flasks. One of these was kept at 37° C., the other at room temperature. Dilutions were prepared daily for eight consecutive days and 1 c.c. of each dilution inoculated into each of five lactose broth tubes. These were examined after three days, and streptococci appeared in all instances in high dilutions. By this method the presence of streptococci even when they occur in very small numbers can be readily demonstrated. Dilutions prepared from fresh milk showed streptococci in chains of immense length. Litmus milk inoculated from these tubes coagulated in typical fashion in 24 to 48 hours, but the long chains had disappeared, and in their stead, diplococci and short chains of three to six members appeared. As the acid fermentation progressed, the long chain formation became more and more scarce, giving way to diplococcus and short chain formation. It appears from this experiment that long chains disappear if these lactic streptococci are cultivated in milk. (See plates.)

(2) Plates in lactose litmus agar were prepared for eight successive days from two flasks of milk from the same lot, undergoing spontaneous fermentation, one at 37° C. the other at room temperature. An approximate enumeration of the colonies of *B. aërogenes* and "*B. lactis acidi*," shows that at initial stages of fermentation *B. aërogenes* multiplies more rapidly than the other type; at later stages, however, the Leichmann type gains ascendency. This phenomenon is more pronounced at room temperature than at 37° C.

(3) A third series of experiments was of the following nature: Ten liters of milk were mixed and evenly distributed in 42 flasks. Thirty-eight of these were sterilized in the Arnold for three successive days, and allowed to stand at room temperature for six days, before being used. The other four flasks were allowed to lopper, two at 37° C., the other two at room temperature. One of the sterilized flasks was

500 C.C. FLASKS OF STERILE MILK INOCULATED WITH PURE CULTURES, VARIOUS MIXTURES OF LACTIC ACID BACTERIA, SOUR MILK AND FLASKS WITH NATURALLY SOURED MILK.

NUMBER OF FLASK	INOCULATED WITH	ODOR	COAGULATION			ACIDITY PER CENT IN LACTIC ACID		Number of Days after Which Highest Acidity Was Reached
			In Days	Homogeneous	Broken up and Whey	At Time When Coagulation Was First Observed	Highest Acidity Reached	
1....	Control	....	....	....	....	....	0.164	1
2....	Pathogenic streptococcus	Sour mild	5	+	—	0.507	0.726	11
3....	" "	" "	5	+	—	0.508	0.773	11
4....	Lactic acid bacterium from sweet milk	" "	3	+	—	0.664	1.004	13
5....	Lactic ac. bact. from separator slime	" "	1	+	—	0.716	1.040	5
6....	Bacillus from sweet milk	Sour and Cheesy	3	—	+	0.541	1.006	11
7....	Bacillus from sour milk	" "	3	—	+	0.619	0.816	8
8....	B. acidi lactici, University of Chicago	" "	4	—	+	0.669	1.149	10
9....	1 c.c. sour old milk for inoculation	" "	1	—	+	0.707	1.130	6
10....	Lactic acid bacter. from N. Y. Station	Sour mild	4	+	—	0.532	1.056	9
11....	Lactic acid bacterium from teat wash water	" "	1	+	—	0.557	0.969	5
12....	Streptococcus from cow feces	" "	7	+	—	0.576	0.790	13
13....	Liquefying streptococcus from milk	Indifferent	2	—	+	0.611	1.141	6
14....	Lactic acid bacterium from separator slime	Sour mild	2	+	—	0.637	0.928	6
15....	Fresh cow feces	Sour and cheesy	2	+	—	0.637	1.065	5
16....	" " "	" "	3	—	+	0.621	1.065	5
17....	1 c.c. milk after 5 days at 37°	" "	3	—	+	0.655	1.240	8
18....	1 c.c. milk after 2 days at room temp.	" "	2	+	—	1.013	1.169	8
19....	Sewage streptococcus	Sour mild	3	+	—	0.532	1.018	8
20....	Pathogenic streptococcus	" "	5	+	—	0.699	0.995	10
21....	" "	" "	5	+	—	0.611	1.135	12
22....	B. aërogenes 1, University of Chicago	Sour mild	5	+	—	0.646	1.014	13
23....	B. coli A	Sour	5	+	—	0.607	1.310	12
24....	B. aërogenes from milk	Sour and cheesy	4	—	+	0.594	1.096	8
25....	Lactic acid bacterium (Russell)	Sour mild	6	+	—	0.628	0.778	12
26....	" " " (Prescott)	" "	7	+	—	0.546	0.546	7
27....	" " " (Marshall)	" "	3	+	—	0.568	0.820	8
28....	" " " from sour milk	" "	3	+	—	0.542	0.734	7
29....	# 24+28	Sour	2	—	+	0.546	1.057	9
30....	B. aërog. + Lact. acid bact. from milk	"	2	—	+	0.546	0.865	9
31....	B. aërog. (22)+ Lactic acid bact. from sour milk	"	2	—	+	0.620	0.987	10
32....	B. coli A + 28	Sour and cheesy	3	+	—	0.681	0.912	6
33....	B. aërog. + sewage streptococcus	Sour	3	—	whey +	0.599	0.925	11
34....	Milk at room temp.	"	2	+	—	0.921	1.175	5
35....	Milk at 37°	Cheesy	1	—	whey +	0.995	3.033	8
36....	Milk at room temp.	Sour	2	+	—	0.908	1.196	7
37....	Milk at 37°	Cheesy	1	—	whey +	0.764	2.846	8
38...	Pathogenic streptoc. at room temp.	Sour mild	2	+	—	0.570	0.691	7
39....	Lactic acid bacteria from separator slime at 37°	Sour mild	2	+	—	0.820	0.873	4
40....	Bacillus from sweet milk at 37°	Sour and cheesy	6	+	—	0.568	0.707	8
41....	# 21+6	Sour and cheesy	2	—	whey +	0.570	0.672	4
42....	# 5+6	Sour and cheesy	2	+	—	0.550	0.725	9

retained as control, the others were inoculated with various pure cultures and mixtures. Five pathogenic streptococci, a streptococcus from sewage, a streptococcus from cow feces, and eleven lactic bacteria of the Leichmann type (the latter including five varieties obtained from different laboratories), four varieties of *B. aërogenes* from milk and one from the laboratory collection, and a culture of *B. coli* recently isolated from human feces, were inoculated in pure culture in sterile flasks. Several flasks were inoculated with mixtures of pure cultures of each group, two with suspensions of cow manure, and three with sour milk.

A study of the results which appear in the accompanying table, demonstrates that the pathogenic streptococci (2, 3, 20, 21), the streptococci from sewage (19) and cow feces (12), the lactic acid bacteria from Professors Harding (10), Marshall (27), Russell (25), Prescott (26), and the lactic acid bacteria from milk (4, 28), separator slime (5 and 14), and teat wash water (11) produce fermentations in milk, which are identical. The coagulum is homogeneous, no whey appears, and the odor is agreeably, but faintly, sour. The range of time required for coagulation varies, but it has been shown that a sojourn in milk increases the power of rapidly coagulating milk.

Bacilli (6, 7, 8, 22, 23) produce a coagulum, which contracts or breaks up and liberates larger or smaller quantities of whey, accompanied by gas formation. The odor is decidedly sour and cheesy.*

Sterile milk, inoculated with sour milk, ferments like naturally soured milk, and produces the same variation of appearance. Suspensions of cow feces (15 and 16) also produce fermentations, which are indistinguishable from naturally soured milk. Occasionally liquefying streptococci are met with (13), which produce high acidity and at the same time dissolve the caseïn.

All flasks were titrated for acidity up to the maximum point, i. e., when three successive titrations gave the same result. The highest acidity was reached by naturally soured milk at 37° C. Milk soured at room temperature, and sterilized milk inoculated with mixtures and feces, fermented at approximately the same ratio. Streptococci of pathological, fecal, and milk origin form a class very similar among themselves in regard to results.

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* Leichmann has shown that his lactic acid bacterium produces almost pure lactic acid, Hueppe's bacillus (*B. aërogenes*) produces large amounts of volatile acids, hence the difference in odor (*Centralbl. f. Bakt.*, Abth. 2, 1896, 2, p. 777).

An interesting fact was brought out by comparing the results of the activity of *B. aërogenes* from milk and a mixture of the same organism with a lactic acid bacterium of the streptococcus type. The bacillus alone produced a finely granular precipitate and abundant gas. The mixture produced much less gas, the gas evolution ceasing after a short period of time. The bacillus was crowded out by the non-gas forming streptococcus and thus gas evolution stopped. Under natural conditions both types of lactic acid bacteria may be concerned in the acid fermentation of milk. Leichmann[1] has called attention to this phenomenon by stating that he isolated the Hueppe type from superficial layers where oxygen is readily obtained, the Leichmann type from deep parts of fermenting milk, the latter preferring anaërobic conditions.

That lactic fermentation varies is a matter of everyday experience. Without apparent cause the condition of the coagulum and the amount of whey separated varies in milks of excellent character. This investigation shows that typical lactic acid fermentations can be produced by inoculation of sterilized milk with lactic acid bacteria of either group, or by a mixture of both. The fermentation proceeds in a substantially similar manner as in the natural process, in spite of the chemical and physical alterations in milk which are unavoidable during sterilization. The various appearances of acid fermentations are brought about by various species of bacteria which may bear different numerical relations to each other, and by the variable degree of multiplication according to the temperature at which fermentation takes place. There is, however, a higher degree of acidity produced in naturally soured milk than in sterilized milk inoculated with these two common lactic acid bacteria either in pure culture or in mixtures. This shows clearly that other influences are at work, and in this connection Marshall[2] has pointed out that other bacteria, especially peptonizing bacteria, exert a favorable influence on the development of lactic acid bacteria.

The lactic acid bacteria of Leichmann, Günther and Thierfelder, Kozai (1), Utz (1), Esten, Schierbeck, and others are described by these writers as follows:

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[1] *Loc. cit.*

[2] *Centralbl. f. Bakt.*, Abth. 2, 1902, 9, p. 313.

They are short rods (?) somewhat more than half as broad as long, are oval or frequently with pointed ends, occur in pairs and chains, and are positive to Gram's stain. They are non-motile, do not liquefy gelatin, have neither spores nor capsules. They ferment dextrose and lactose with formation of acid but not of gas. They grow sparingly in ordinary media, much better if dextrose or lactose is added. In stab cultures there is little or no growth on the surface. They grow well in absence of oxygen, some preferring anaërobic conditions. They acidify milk rapidly, form a homogeneous coagulum and decolorize litmus. In non-proteid media no growth is observed. Colonies are small, shapeless and appear preferably in the depth of the jelly.

The characteristics are in perfect accord with those resulting from the studies described. When lactic acid bacteria of the Leichmann type are compared with pathogenic, fecal, or sewage streptococci, it is impossible to detect a difference as far as cultural characteristics have been observed. A careful perusal of v. Lingelsheim's summary of characters of streptococci in Kolle and Wassermann's *Handbuch* demonstrates conclusively that there is no salient difference between recognized streptococci and lactic acid bacteria of the Leichmann type. It may, then, be confidently asserted that there is no organism known which deserves the names *B. acidi lactici* or *B. lactis acidi.* We can divide the common lactic acid bacteria into two groups. The first group are true bacilli, those described by Pasteur, Hueppe, Clauss, Grotenfelt, Schardinger, Kozai (2), and Utz (2). They are varieties of *B. aërogenes* and may be termed *B. aërogenes var. lacticus.* The second group includes the lactic acid bacteria described by Leichmann, Günther and Thierfelder, Kozai (1), Utz (1), Esten, Schierbeck, and others—all these are called bacilli by the authors—and Grotenfelt's, Weigmann's and Freudenreich's streptococci.[1] Kruse[2] has suggested the relation of the latter group to streptococci although no experimental evidence is reported, and proposed the appellation *Streptococcus lacticus.*

A general summary of the results leads to the following conclusions:

1. *Bacillus acidi lactici* is a myth. The ordinary bacteria producing lactic acid fermentation in milk are *B. aërogenes var. lacticus,* and *Streptococcus lacticus.* The possibility of *B. coli* and other forms participating in milk fermentation is not excluded.

2. *Streptococcus lacticus* agrees in morphological, cultural, and coagulative properties with pathogenic, fecal, and sewage streptococci.

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[1] See MIGULA, *System der Bakterien,* 1900, 2, pp. 18, 39, 42.

[2] *Centralbl. f. Bakt.,* Abth. 1, 1903, 34, p. 737.

3. *Streptococcus lacticus* can be detected in cow feces, on the external surfaces of cows, and in milk at all stages of subsequent handling.

4. Souring of milk is caused by co-operation of both groups of lactic acid bacteria and may be aided by some peptonizing bacteria always present in market milk.

5. Gas is produced by *B. aërogenes var. lacticus*, but as a rule this organism is held in check and ultimately stopped by the presence and final ascendency of *Streptococcus lacticus*.

6. Acid is produced during lactic acid fermentation of milk by both classes of organisms to a marked degree. *B. aërogenes var. lacticus* is more sensitive to the presence of acid than *Streptococcus lacticus*. This results in the presence of *B. aërogenes* in large numbers in initial stages of fermentation, *Streptococcus lacticus* becoming master of the field in terminal stages.

7. Artificial lactic acid fermentation in sterilized milk can be produced by inoculation of pure cultures of bacteria of either group, or better by the two groups combined.

8. Since *Streptococcus lacticus* is invariably present in market milk and in fresh milk collected with good precautions, the sanitary significance of streptococci in market milk will need further investigation.

In conclusion I wish to thank Professor Edwin O. Jordan, under whose direction this work was carried on, and Dr. Norman Mac L. Harris for valuable advice and encouragement during the progress of the work.

DESCRIPTION OF PLATES.

PLATE 3.

Streptococcus pyogenes from tonsil in scarlet fever.

FIG. 1.—From serum broth.
FIG. 2.—From lactose broth.
FIG. 3.—From litmus milk.

PLATE 4.

Streptococcus lacticus from the Massachusetts Institute of Technology.

FIG. 1.—From serum broth.
FIG. 2.—From lactose broth.
FIG. 3.—From litmus milk.

PLATE 5.

Streptococcus lacticus from Chicago milk.

FIG. 1.—From serum broth.
FIG. 2.—From lactose broth.
FIG. 3.—From litmus milk.

PLATE 3.

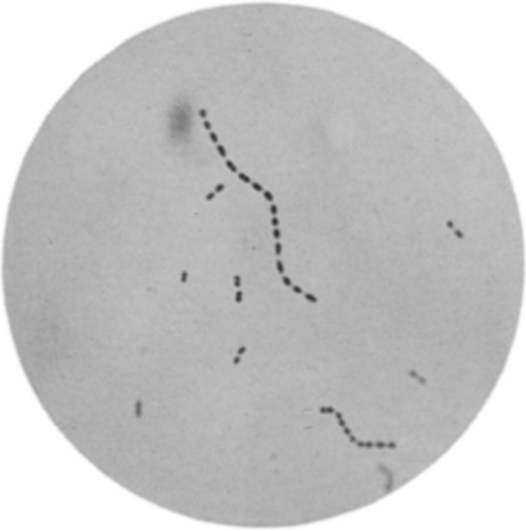

FIG. 1.

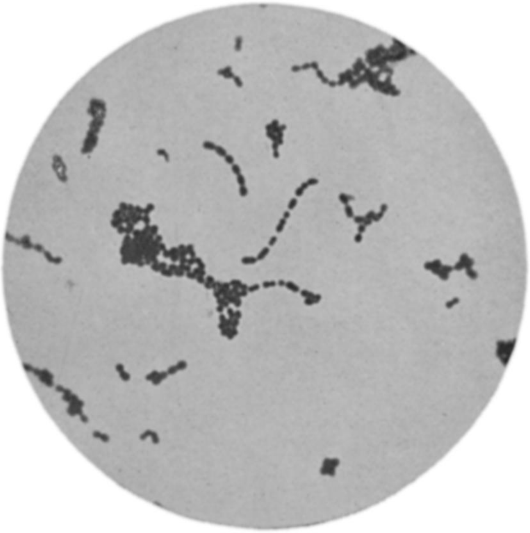

FIG. 2.

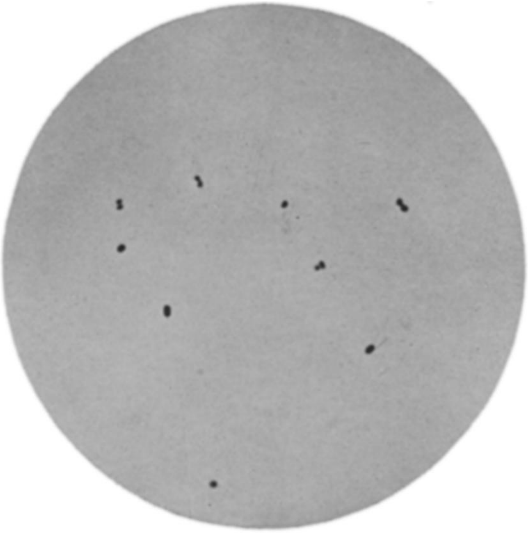

FIG. 3.

PLATE 4.

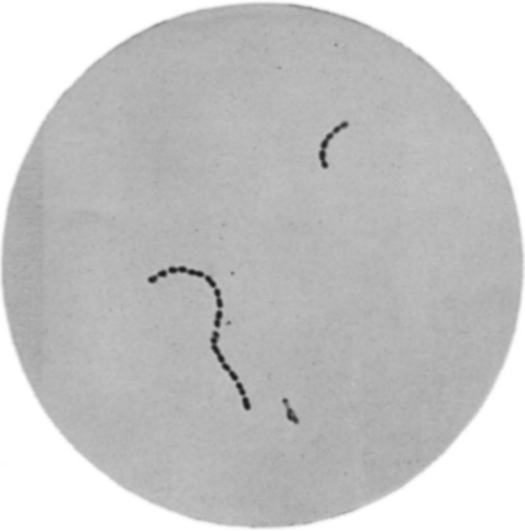

Fig. 1.

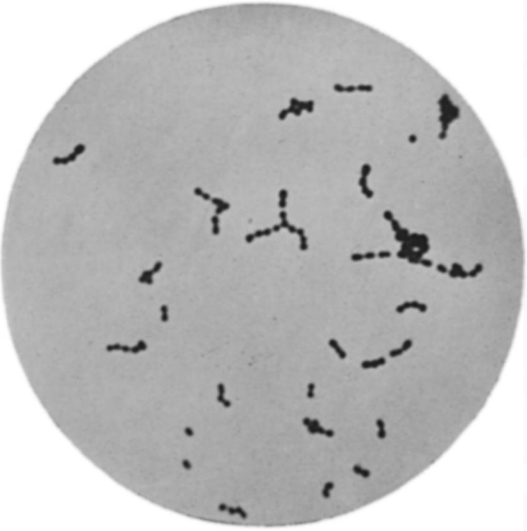

Fig. 2.

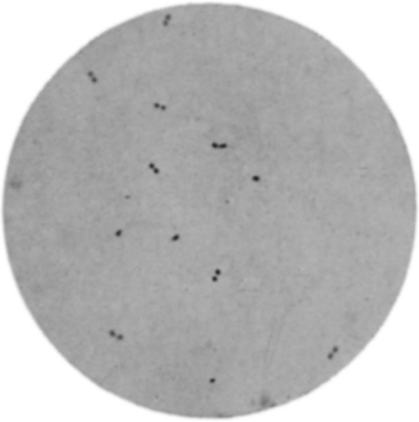

Fig. 3.

PLATE 5.

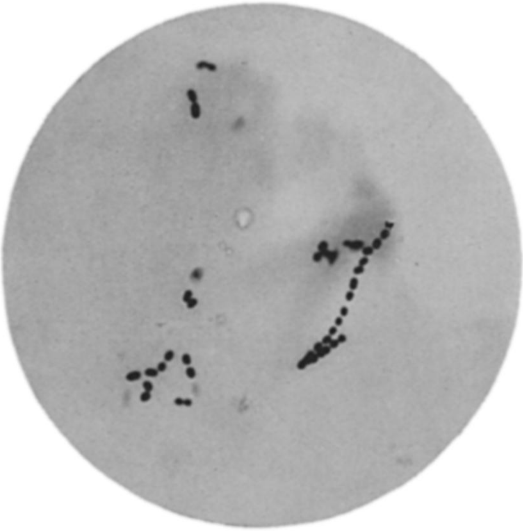

FIG. 1.

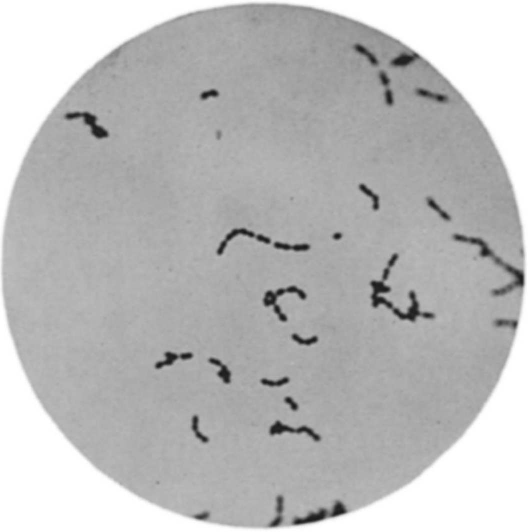

FIG. 2.

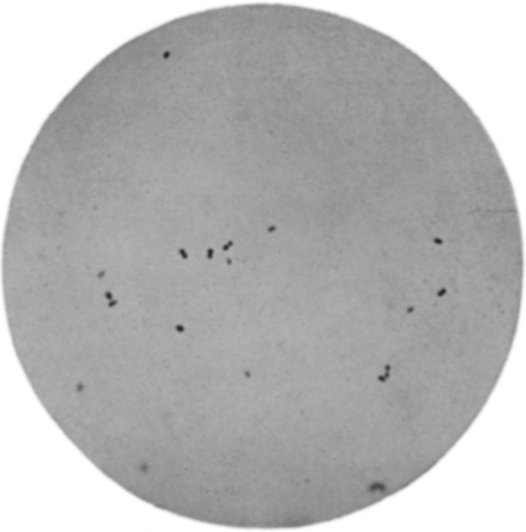

FIG. 3.